Report of Dr. Cole and Dr. Avery (assisted by Drs. Beeson, Dubos, Goebel, Goodner, Hoagland, Horsfall, Hotchkiss, MacLeod, Reeves, Stillman and Thompson).

Serum treatment of pneumonia (MacLeod, Goodner, Beeson, and Hoagland) Since the use of unconcentrated antipneumococcus rabbit serum was begun two years ago, 75 cases comprising infections due to 11 types of pneumococcus have been treated in the hospital. Of these 7 have died, a mortality rate of 9.3%. Until fairly recently adequate amounts of Type III antipneumococcus rabbit serum have not been available and if all cases due to this type are excluded, the mortality rate falls to 5.26%.

An attempt has been made to obtain a larger number of Type III patients in order to test the usefulness of antipneumococcus rabbit serum in infections due to this type. It would appear that in a certain proportion of cases, adequate amounts of serum given early in the disease will induce rapid disappearance of the acute symptoms in much the same way as occurs with pneumonia due to other types. The greater variability in the course of Type III pneumonia, however, particularly in respect to infections occurring in young and old individuals makes the results of therapy difficult to assess. Thus, in cases occurring before the sixth decade, the disease tends naturally to run a much less malignant course.

The skin test with the homologous type specific capsular polysaccharide has been extended to patients with infection due to Pneumococcus Types II, III, and VIII in addition to Type I and has proved in most instance to be an accurate means of determining when sufficient serum has been given. The difficulties associated with this test are due to occasional false positive or false negative reactions. The confusion resulting from false positive reactions can be avoided, as was previously found with the Type I polysaccharide skin test, if the patient's reactivity to the intradermally in-

jected polysaccharide is determined before the injection of serum is begun. The information gained from frequently repeated skin tests has made it possible to give the optimum therapeutic dose of serum over a shorter period following admission than was possible previously.

The length of time consumed in administering the necessary therapeutic amount of serum has a striking effect upon the duration of acute symptoms, since termination of the disease by crisis can occur only when sufficient antibody has been given, or the patient's own defense mechanism has gained the upper hand. The traditional method of administering antipneumococcus serum has been that of divided doses over a variable period of time, until evidences of subsidence of the acute process had occurred, or a positive skin test to the homologous capsular polysaccharide had appeared. During the past year an attempt has been made to administer the full therapeutic dose of serum in a single intravenous injection as soon as possible following the admission of the patient to hospital. 70 cases, distributed among nine types of pneumonia have been so treated with unconcentrated antipneumococcus rabbit serum. In these cases the average elapsed time from the beginning of therapy until the termination of the acute symptoms has been only 8.6 hours. The uniform success of this method of serum administration is conditioned by the need of accurate knowledge of the antibody content of the serum used, and the severity and extent of the infection from which the patient is suffering.

Antipneumococcus rabbit serum (Goodner). Reports submitted in previous years have dealt with the rationale of antipneumococcus rabbit serum in the therapy of human lobar pneumonia and with the first results of clinical trial. The present account may be regarded as a progress note. The present status of the problems which are now in the foreground is considered with reference to changes which have occurred within the year.

Clinical results: Sera prepared in this laboratory have been used in the treatment of lobar pneumonias associated with Pneumococcus Types I, II III, IV, V, VI, VII, VIII, IX, XIV, XVIII, XIX, and XXVIII. Sera for certain other types are now available. Except with Type III the results have been exceptionally good and have justified the theoretical deductions which led to its use.

With Type III pneumonias the results are still regarded as inconclusive. It is difficult to produce serum of good titer and the supply has been somewhat limited. It is a curious contradiction that certain cases with positive blood cultures have responded in excellent fashion to the administration of this serum while others admitted to the hospital with negative blood cultures have not responded and the course of the disease has been terminated by death. Certain of these therapeutic failures have been associated with conditions beyond the scope of serum therapy but there remained a curious paradox. Observations made during the course of the present pneumonia season may, however, furnish the clue necessary for the solution of this problem. A large number of persons are normal carriers of this type of pneumococcus and it seems to be associated with bronchitis in some instances. It seemed not unlikely, therefore, that while this type of pneumococcus might be predominant in the sputum another type might serve as the actual infecting agent. A somewhat similar instance has been observed in the experimental animal, viz.; Type III pneumococci are not highly virulent for the rabbit but if the tissues are prepared, as for example by extracts of other types of pneumococci, the Type III becomes exceedingly virulent. Consequently a vigorous search has been made for type pneumococci other than III in these clinical cases. In seven instances, or approximately 75 per cent of the so-called III cases, another type pneumococcus has been discover

and five of these cases have been treated as of the concomitant type. Th cases have recovered. Two cases of mixed infection were treated as Type and both died. It is undoubtedly true that Type III can infect alone and that it can cause death but the foregoing observations bid fair to shed c siderable light on the apparent failure of the specific serum to benefit certain proportion of these cases. The solution probably resides in most careful typing and the application of this rule: When Type III and one o pneumococcus type are present in the sputum the other type pneumococcus s be regarded as the inciting agent.

Toxicity of rabbit serum: Methods have been developed which gr ly reduce and in most instances entirely eliminate the toxicity associate with raw antipneumococcus rabbit serum. These methods are simple and inexpensive and it is gratifying to report that other laboratories which ha employed these methods have been uniformly successful in the production c non-toxic serum.

Standardization of serum: The question of standardization of antipneumococcus serum is much too complicated to elaborate in this repor It may be said, however, that the only hope for logical dosage is that a satisfactory method of standardization be achieved. Great progress has been made in this direction and this laboratory is collaborating with sta and federal agencies in a plan leading to the adoption of suitable standa and methods for standardization. The sera in this laboratory are evalua on the basis of actual antibody content.

Amounts of serum required to bring about recovery: With accura evaluation of serum potency it is now possible to study the factors whic in one patient require that a very large amount of serum be used while i another permit adequate therapy with small amounts. It is obvious that amount is determined partly by the severity of the infection but the cap

of the individual to utilize the passively conferred antibodies is even more important. This laboratory has developed a considerable experience in the evaluation of the so-called host factors in experimental infections. This work had been undertaken with the idea that it might some day become useful in the clinic. Much to the satisfaction of all concerned a direct application of these findings has been discovered and it may be expected that within a few weeks an accurate dosage scale will be available for use in most cases of lobar pneumonia.

The use of calcium chloride in the relief of chills following antipneumococcus serum therapy (Beeson and Hoagland). The occasional occurrence of chills following serum administration has to some extent interfered with the widespread adoption of serum therapy in pneumonia. Because the inadequacy of the palliative measures employed in the treatment of such chills, an attempt was made to find an agent which would alleviate them. In a consideration of chemical substances which might prevent or alleviate chills, calcium chloride, owing to its effect in relaxing smooth muscle, seemed a good possibility.

To date, 10% calcium chloride solution in amounts from 5 to 20 cc has been administered intravenously to 18 patients, during chills following antipneumococcus serum therapy. The results obtained have been encouraging In 11 cases (61%) there was complete relief, subjective and objective. In 7 cases (39%) there was only partial, or transient relief. Five of the patients in the latter group had received epinephrine during the serum administration and it is possible that this accounted in part for the poor result obtained with calcium therapy, since calcium and epinephrine are known to be antagonistic. Calcium chloride is not considered a dangerous therapeutic agent, except in those patients who have received digitalis.

Studies are now in progress as to the mechanism of this action of calcium, also in regard to the possibility of a similar effect in chills due to other causes.

Serum lipids in pneumococcal pneumonia (Hoagland). That blood lipids play an important part in certain acute infectious processes is increasingly evident as more exact technical procedures for fat estimation become available. Of these, cholesterol has received the most emphasis. Lately, however, variation in the total lipid pattern in various diseases has engaged considerable attention.

Cholesterol has long been known to possess marked antihemolytic properties against oleates, pneumococcus hematoxin and tetanolysin, and both early and recent workers have found a marked drop in the cholesterol level during acute infections, with return to normal values during early convalescence.

The early work of Christian in showing vast stores of oleic fatty acids in the stage of grey hepatization, and the work of Lamar in showing accelerated lysis by immune sera of pneumococci treated with minute amounts of sodium oleate, attach considerable importance to the role of lipids in the recovery from pneumococcal pneumonia.

The present study was undertaken in order to gain some idea of the blood lipid pattern in pneumococcal pneumonia, and to correlate, if possible, the change in blood lipids with the onset, course, and convalescence in the disease.

Observations presented in this summary were made on the serum lipids of 25 patients with lobar pneumonia, with analyses at three day intervals, covering the disease in most instances from its onset, through its course to convalescence. Total lipid carbon values have been obtained in all cases, together with a smaller series in which the unsaturation, as

reflected by the serum iodine uptake, and cholesterol values, free and combined, have been determined.

Micro-gasometric technique, after the method of Kirk, Page, and Van Slyke, modified by Folch-Pi, was used throughout. Serum iodine absorption determinations were carried out according to the method of Page, Pasternak and Burt.

Analysis of data reveals in all cases a marked drop in serum lipid carbon, cholesterol and unsaturated fatty acids during the onset of pneumococcal pneumonia, with a return to normal during convalescence. The most marked drop appears to be in the cholesterol ester fraction, which in many instances falls to less than half the normal or convalescent level. Free cholesterol values are not significantly altered, which is particularly interesting in the light of recent work by Sperry who was able to show a remarkable constancy in the ratio between free and ester cholesterol in normal individuals over an extended period of time. The fall in serum unsaturation appears to parallel the drop observed in total lipid carbon and cholesterol ester, and to return quite as promptly to the convalescent level.

Although data are incomplete at present, it would appear that the period of hypolipemia in pneumonia is considerably shortened by the early institution of serum therapy.

As an example of the changes observed in the serum lipids during the onset, course and convalescence of pneumonia, analyses in two cases are presented as follows:

| Case | Day of disease | Total lipid carbon | Cholesterol Free | Ester | Total | Unsaturation Mg. Io/100 cc. serum |
|---|---|---|---|---|---|---|
| Early serum administration | 2 | 412 | 40 | 140 | 180 | 292 |
| | 5 | 700 | 38 | 71 | 109 | 240 |
| | 8 | 512 | 42 | 180 | 222 | 362 |
| | 11 | 418 | 46 | 195 | 241 | 412 |
| Untreated | 2 | 387 | 30 | 152 | 182 | 320 |
| | 5 | 274 | 32 | 95 | 127 | 215 |
| | 8 | 238 | 28 | 70 | 98 | 208 |
| | 11 | 277 | 28 | 122 | 150 | 290 |

Certain properties of the C-reactive substance present in the blood during pneumonia and other infectious diseases (Avery and MacLeod). Earlier studies from this laboratory have shown that during the active stage of lobar pneumonia there is demonstrable in the blood, a substance which yields a precipitate when a dilute solution of the "C" carbohydrate of Pneumococcus is added to patients' serum. The precipitable substance has been detected in serum obtained within 4 to 12 hours after the initial chill. The precipitation reaction remains positive throughout the course of active disease and becomes negative with the onset of recovery. Patients in whose blood the reactive substance is demonstrable develop a characteristic skin reaction following the intradermal injection of 0.1 mg. of the test carbohydrate. In a large series of patients studied, the results of the cutaneous and precipitation tests are in close agreement and both reactions parallel in striking manner the clinical course and outcome of the disease. Thus, the presence of this substance in the circulation during the acute disease and its absence in convalescence are in turn correlated with the activity and termination of the disease process in man.

It was soon found, however, that the principles involved in these reactions are not restricted merely to pneumococcal infections. An identical substance, or at least one chemically so similar as to be equally

reactive with the same test carbohydrate, is present in the blood during the acute phase of certain infectious diseases caused by agents other than Pneumococcus. For example, in rheumatic fever, bacterial endocarditis, pulmonary tuberculosis and a number of other infections, there exists in the blood a substance which is serologically the same as that found in pneumonia. It appears, therefore, that the reactions described are not specific with reference to the etiological incitant of the infection but represent rather a mutual interaction of a selective carbohydrate with the reactive substance common to many infections of unrelated etiology. This substance possesses the property of combining with the test carbohydrate to form a precipitate. No other bacterial sugar thus far tested has been found to precipitate the material in reactive serum. It will be recalled that the "C" carbohydrate is present in the cell body of all forms of pneumococci irrespective of their type derivation. Although chemically less well defined, this carbohydrate differs markedly from the capsular polysaccharides in containing phosphorus and pentose but no uronic acid constituent in the molecule.

Attempts are being made to isolate the reactive substance from serum and to determine its chemical nature. On fractional precipitation of patients' serum with ammonium or sodium sulphate, the active material is carried along with the serum albumin, the globulins being wholly inert. From a solution of the albumin it is precipitated together with the portion of normal albumin removed by 2/3 saturation. On removal of salts by dialysis at neutral or slightly alkaline reaction, the active substance precipitates from the solution of inert albumin. The material recovered is active with "C" and has the following properties; it is soluble in dilute alkali and physiological saline; insoluble in water and dilute acids; it is readily denatured and inactivated at hydrogen ion concentrations more acid than pH 6.

The extreme lability of the active substance differentiates it from the normal blood proteins but renders difficult its isolation and purification in active form.

From reactive human serum the substance can be quantitatively precipitated by the addition of optimal amounts of the carbohydrate. On the basis of its known properties, techniques are being devised to dissociate the active material from the "C" precipitate. The formation of a precipitate is conditioned by the presence of calcium in the reaction-system, since no precipitation occurs on adding the carbohydrate to reactive serum from which the calcium has first been removed. On the readdition of minute traces of $CaCl_2$ to such serum, the active substance again precipitates in the presence of the test sugar.

The C-precipitable substance has not yet been obtained in a sufficiently purified form to justify any conclusion as to its nature. However, on the basis of its nitrogen content (14%) and certain other properties, it is tentatively regarded as a protein and one not normally found in the blood of healthy individuals.

Work on the purification of this interesting substance and a study of its chemical and antigenic properties are being actively pursued. One would like to know its origin and fate in the animal body; its possible significance in relation to certain manifestation of disease; and the chemical groupings involved in the precipitation reaction with "C". The interpretation of the precipitation and skin reactions in terms of antigen-antibody union is difficult for the following reasons: the reactive substance is demonstrable not only in pneumonia but in diseases not associated with pneumococcus infection; it is present in the blood early in the course of disease, long before specific antibodies are demonstrable, and it disap-

pears from the circulation during convalescence, a time when antibodies are usually found in highest concentration. Thus, the time of appearance and disappearance of this substance with respect to the occurrence of known antibodies is completely reversed; this together with the lack of specificity, the association of the reactive substance with the albumin rather than with the globulins of serum, and the importance of calcium in the precipitation reaction are facts not easily reconciled with the orthodox conceptions of antigen-antibody phenomena.

Pneumococcal carrier state in nephrotic children (MacLeod and Farr) Most of the deaths occurring in children with nephrosis are due to pneumococcal peritonitis. A study has been made of the pneumococci carried in the naso-pharynx of such cases, in order to determine whether peritonitis is caused by the strain or strains of pneumococcus carried in the patient's throat or whether it is due to the acquisition from a carrier of an invasive strain of pneumococcus. The first supposition has been found to be most generally true.

In all the cases which developed peritonitis the same type of pneumococcus had been recovered from the throat and the peritoneal cavity. The longest period from the first isolation of a strain of pneumococcus from the throat until peritonitis due to the same type occurred, was three months.

Although children who recover from peritonitis may carry the invading strain in their throat for as long as a year afterward, in some instances specific agglutinins may not be demonstrable in the blood serum. However, the development of specific agglutinins does not guarantee freedom from peritonitis, since the serum of one patient who died showed specific agglutinins against the invading organism on the day the fatal infection began.

Children who do not develop peritonitis, and who recover from nephrosis, tend to show more variation in the types of pneumococci carried

in the nasopharynx. Thus an acquired strain may be rather quickly lost or replaced by a different strain.

The role of nucleic acid in the structure and immunological behavior of the pneumococcus cell (Dubos and Thompson). The preparation of a ribonuclease from animal tissues and its effect on heat-killed pneumococci. It has been described in a previous report that leucocytes contain a heat resistant enzyme capable of attacking killed pneumococci. The enzyme has been purified by a technique based on the following properties; a) it is soluble in 50% acid acetone, but precipitates from this medium on addition of more acetone, b) it can be subjected to boiling temperature between pH 3 and pH 6.5 without any loss of activity. The purest preparation so far available contains 12% Nitrogen and 0.55% Phosphorus.

The only soluble substrate found to be attacked by the enzyme is ribonucleic acid, which it depolymerizes.

When heat-killed pneumococci are resuspended at neutral reaction in the presence of the enzyme, they slowly lose their affinity for the basic dyes although this change is not accompanied by any evidence of cellular disintegration. Under these conditions the killed cells of encapsulated pneumococci lose the power to stimulate in rabbits the production of the type specific antibodies directed against the capsular polysaccharide of the cells; in other words the ribonuclease inactivates the capsular antigen of virulent pneumococci.

The extraction of ribonucleic acid and of a ribonucleoprotein from pneumococci. Although pneumococci are killed within a few seconds in an acetate buffer at pH 4.2, they retain in this acid medium their Gram positive character; when the same cells, however, are separated from the acetate solution and resuspended at neutral reaction, they slowly undergo a change from

the Gram positive to the Gram negative state and at the same time lose their affinity for the basic dyes. Two of the substances which are released in solution in the course of this change, have been separated and identified. One fraction is soluble in dilute acetic acid but insoluble in HCl. it contains 14% N and 7.75% P and is decomposed by the ribonuclease described in the preceding section of this report. This substance is a ribonucleic acid.

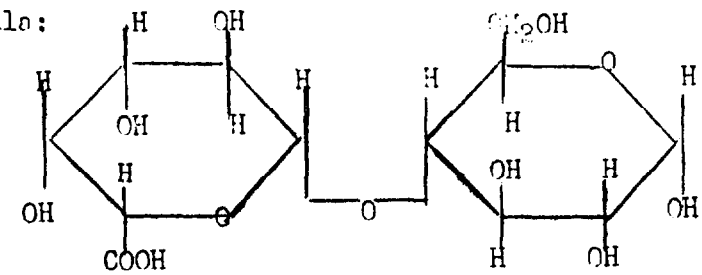
Another fraction is precipitated at pH 4.2 and remains insoluble in stronger acids; it contains 14% N and 4% P. The high phosphorus content is due to the presence of ribonucleic acid in the molecule. This nucleic acid is combined with a protein which is deficient in aromatic amino-acids and in sulfur and which may therefore belong to the group of protamines or histones.

The release in solution of nucleic acid and of nucleoprotein, whic takes place when pneumococci killed at pH 4.2 are resuspended in neutral solution, is a phenomenon common to both R and S variants, irrespective of type derivation. In the case of the encapsulated cells, there is observed at the same time a loss of the ability to stimulate rabbits to produce the specific antibodies directed against the capsular polysaccharide. It appears, therefore, that when the ribonucleic acid of the Pneumococcus cell is attacked by ribonuclease, or when an injury to the cellular structure allows it to diffuse into the medium, the capsular antigen is at the same time inactivated. Nothing is known, however, of the nature of this relationship.

It may be mentioned here that a study is being made of the immunological properties of the nucleoprotein fraction released in solution when the cell changes from the Gram positive to the Gram negative state; definite evidence has been obtained that this fraction, when injected into experimental animals, can produce immunity to infection with virulent pneumococci; this protection is not type specific and the antigen can indeed be prepared from

rough as well as encapsulated pneumococci. So little is known of this phenomenon, however, that a detailed description is not warranted at the present time.

Structure of Type III pneumococcus polysaccharide and aldobionic acid (Goebel, Reeves and Hotchkiss). The capsular polysaccharide of Type III Pneumococcus is a macromolecule constituted from units of an aldobionic acid linked in glycosidic union. In addition to its property of conveying virulence and type specificity upon the organism from which it is derived, the capsular polysaccharides, when injected into certain species, induce an active antipneumococcal immunity. Because of these important biological properties we have found it desirable to establish the constitution of the aldobionic acid from which the polysaccharide of Pneumococcus Type III is constituted. In collaboration with Dr. Hotchkiss we have shown that the aldobionic acid is 4 $\beta$-glucuronosido-glucose and has the following structural formula:



Since this sugar acid is the uronic acid of cellobiose, the disaccharide unit from which cellulose is constituted, we have termed the Pneumococcus Type III aldobionic acid cellobiuronic acid. The relationship of the capsular polysaccharide to cellulose is obvious.

The position of glycosidic union of aldobionic acid units to one another in the intact polysaccharide molecule is being investigated with the assistance of Dr. Reeves. When the carbohydrate is methylated with methyl sulfate and alkali, a fully methylated derivative is obtained which still retains the property of reacting in antipneumococcus serum. This fact

reaffirms the concept expressed in earlier reports that the union of polysaccharide to homologous antibody is primarily through the carboxyl group of the former. The identification of the products of hydrolysis of the methylated polysaccharide is under investigation and will yield information definitely establishing the complete structure of the specific polysaccharide itself.

Synthetic antigen containing cellobiuronic acid (Goebel). Although antipneumococcal immunity can be induced in certain species, including man, by immunization with the intact specific polysaccharide, it is now known that immunity may also be induced by chemically modified and depolymerized forms of the carbohydrate. It is of great interest, therefore, to ascertain whether immunity to Type III Pneumococcus infection can be induced by the immunization of animals with the aldobionic acid unit of the polysaccharide molecule. Consequently a method for the synthesis of aldobionides has been devised, and used in the successful synthesis of the p-aminobenzyl glycoside of cellobiuronic acid. This derivative, when combined with protein by means of the diazo reaction has yielded an artificial cellobiuronic acid-protein antigen which exhibits many of the serological characteristics of the Type III polysaccharide itself. The antigen precipitates both in antipneumococcus Type III horse and rabbit sera in high dilutions, but not in the normal sera of these species. Experimental animals are being immunized with the antigen and the results will be reported later.

The nature of fowl prothrombin (Schoenheyder and Goebel). Deficiency of vitamin K causes a hemorrhagic diathesis in chicks which is accompanied by a disappearance from the plasma of the clotting factor, prothrombin. In collaboration with Dr. Fritz Schoenheyder an investigation concerning the mechanism underlying this alteration in physiological function was initiated last July through a study of the chemical nature of the pro-

thrombin of normal fowl plasma and a comparison of this plasma fraction with that derived from the plasma of vitamin K deficient birds.

A quantitative method for the estimation of prothrombin was devised, a procedure which was used to great advantage in studying the chemical fractionation of the active agent. By fractionating fibrinogen-free plasma at 0° with ammonium sulfate it is possible to obtain the prothrombic activity in the fraction insoluble in 75-65 per cent saturation. Further fractionation with neutral salt solutions is not feasible. However, adsorption and elution of this material on colloidal gels results in a sharp separation of inert protein from the active principle. On the basis of nitrogen content it is possible to obtain prothrombin solutions with an activity nearly one hundred fold greater than that of the plasma from which it was derived. Solutions of prothrombin thus isolated are quite stable above pH 7.0 and very unstable in the acid range. Although fowl prothrombin, as we have obtained it, is probably not a pure chemical entity, yet from its chemical characteristics, its thermolability, and its susceptibility to enzymatic hydrolysis, the substance appears to be a protein endowed with certain unique chemical and biological characteristics which distinguish it sharply from other plasma proteins. Dr. Schoenheyder's return to Denmark in January terminated the research at this juncture.

Preparation and use of ferrous gluconate in hypochromic anemia (Reznikoff and Goebel). In collaboration with Dr. Paul Reznikoff of the Department of Medicine, Cornell Medical College, we have attempted to prepare water soluble iron compound which can be administered parenterally. A therapeutically effective and readily assimilable compound of iron is greatly to be desired in conducting studies on hemoglobin since parenteral administration eliminates the uncertainty of absorbtion regeneration. It is believed that the local tissue necrosis caused by the injection of inorganic

salts, as well as the gastro intestinal distress which frequently accompanies their oral administration, may be attributed to their capacity to act as protein precipitants. Ferrous gluconate, the iron salt of the polyhydroxy organic acid glucuronic acid, does not precipitate protein because the iron is in a unionizable complex combination with the carboxyl and hydroxyl groups of the hexonic acid. A practicable and inexpensive method for the production of crystalline ferrous gluconate has been devised. Ferrous gluconate compares very favorably with inorganic iron compounds in giving rapid and marked reticulocyte, red cell and hemoglobin responses both in anemic albino rats and in humans suffering from hypochromic anemia. Patients showing toxic reactions to other iron compounds were able to tolerate ferrous gluconate without any undue distress. In patients who receive ferrous gluconate intramuscularly no systemic and only rare and mild local reactions occurred. In view of the efficacy of the oral and parenteral administration of ferrous gluconate and its lack of toxicity the drug possesses properties which render it of value in the treatment of hypochromic anemia.

Viability of pneumococci in dried sputum and blood (Stillman) In continuing the earlier epidemiological studies on the mode of dissemination of the disease-producing types of pneumococcus, experiments have been carried out to determine whether these organisms can survive for long periods of time in dried sputum under conditions simulating those encountered outside the body.

Small quantities of sputum freshly obtained from patients with known types of pneumonia were placed in a series of test tubes stoppered with cotton plugs; one set of tubes was placed at room temperature in diffuse daylight and the other in the icebox at a temperature of about 40°F. At intervals, duplicate specimens of the sputum which had dried at different temperatures and under varying conditions of humidity and light were emulsified in broth and injected into mice. If the virulent pneumococci originally

present in the sputum remained viable in the dried specimen, the inoculated mouse died of infection and pneumococci of the homologous type were recovered in cultures of the animal's blood at autopsy.

In several instances Type I pneumococci remained viable in dried sputum after exposure for 16 weeks at room temperature: Type II after 17 weeks; Type III after 20 weeks. Despite the great variations in the number or organisms present in individual specimens of sputum, and the marked differences in the physical and chemical properties of the various samples, it may be conservatively stated that the average time of survival of Type I organisms in dried sputum is 4 weeks, Type II 6 weeks and Type III 8 weeks.

The length of time during which pneumococci remained viable in specimens of sputum stored in the icebox is considerably longer. Under these conditions Type I pneumococci were recovered from specimens after 15 weeks, Type II 26 weeks, and Type III 16 weeks. In one instance Type II pneumococci were found to be viable in specimens of sputum which had been stored in the icebox for 41 weeks.

The results indicate that under these experimental conditions pneumococci can remain viable and virulent for long periods of time in dried sputum and the findings suggest that these organisms may survive and retain their disease-producing properties under the more rigorous conditions of the outside world for longer periods of time than has generally been supposed.

Similar studies are under way to determine the survival and death rate of pneumococci in dried rabbit's blood. To defibrinated blood, measured quantities of pneumococci of the different types were added, and the inoculated specimens were allowed to dry under conditions similar to those used in the experiments with sputum. Types I and II pneumococci have been recovered from specimens of dried blood which had been kept for two months

exposed to the air under the conditions of temperature, light and humidity prevailing in the laboratory. However, specimens examined after 2 months failed to yield viable organisms. On the other hand Type III pneumococci under identical conditions have remained viable and virulent up to the present time, 8 months after the initial observations.

The production of experimental osteomyelitis in rabbits by intravenous infection with Staphylococcus aureus (Thompson and Dubos). In the course of experiments designed to compare the virulence of different strains of Staphylococcus in rabbits, it was observed that several animals developed well characterized bone lesions following the intravenous injection of a certain strain of Staphylococcus aureus. In view of the conflicting statements in the literature concerning the possibility of producing staphylococcal osteomyelitis in experimental animals, an attempt was made to understand the conditions under which rabbits respond to the injection of this strain of staphylococcus by the development of bone inflammation.

The virulence of the strain was raised by animal passage and especially by isolation from a bone abscess produced in a rabbit by the injection of a large amount of culture. In most of the experiments an attempt was made to produce in the rabbits a low degree of immunity by injection of suspensions of killed staphylococci, prior to infection, in the hope of thereby obtaining a relatively prolonged course of the infection so that macroscopically recognisable lesions might develop before the death of the animal.

Young rabbits were injected intravenously with small doses of culture and were observed daily for the development of any swelling, tenderness or loss of function in the limbs; in some of the experiments x-ray photographs were taken of the animals before death, and also of any inflamed bones after removal from the body at autopsy. At the autopsies particular

attention was paid to the condition of the joints in the hope of determining whether lesions in the metaphysis of the long bones were due to a primary osteomyelitis or to a spread from a primary purulent arthritis.

31 animals in all have been used. 9 of these animals died shortly after they had been infected before sufficient time had elapsed to allow the development of bone lesions recognisable with the naked eye. Of the 22 animals in which the infection assumed a subacute course lasting from 1 to 3 weeks, 18 or 81.8% showed definite macroscopic evidence of bone inflammation. The bone lesions varied from mere softening and hyperaemia with multiple subperiosteal bleeding points, to advanced abscess formation with suppuration and extensive bony destruction. In cases where frank suppuration and bony destruction were absent, microscopic examination of stained films of the bone marrow beneath the inflamed region showed in every case the presence of Gram-positive cocci and of an inflammatory reaction. 55 bone lesions were present in the 18 animals; of these 47 occurred in the metaphysis of the long bones, yet only 8 were associated with a purulent arthritis.

Several points of interest have emerged suggesting a close similarity between the experimental condition and staphylococcal osteomyelitis in children. Firstly, the characteristic predilection of the organism to produce inflammation in the metaphysis of the long bones; secondly, the relative infrequency of spread of a metaphysial abscess through the epiphysis into the neighboring joint cavity and, thirdly, the pyrexial course of the infection in the acute stage, with a lapse into a chronic condition of progressive suppuration, if the animal survive the acute stage.

The results of the work indicate that it is possible to produce consistently inflammation of the bones of rabbits by the mere intravenous injection of a suitable strain of staphylococcus without resorting to any elaborate operative technique designed to localise the organisms in the bones

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